RESPONSE TO OFFICE ACTION

A. Status of the Claims

Claims 2-12 are amended to correct a typographic error. No new matter is added. Claims 1- 16 are now pending and are presented herein for reconsideration.

B. Rejection under 35 U.S.C. § 103

1. Rejection Over Bowen *et al.* in view of Zhong *et al.*, Weeks *et al.*, Poehlman *et al.*, and Cheng *et al.*

The Action rejects claims 1-16 under 35 U.S.C. § 103(a) as being obvious over Bowen *et al.* (U.S. Patent 5,736,369) ("Bowen"), in view of Zhong *et al.* (*Planta* 187: 483-489, 1992) ("Zhong"), in view of Weeks *et al.* (*Plant Physiol.* 102: 1077-1084, 1993) ("Weeks"), in view of Poehlman *et al.* (Molecular Biology: Application in Plant Breeding, Chapter 8, *In* Breeding Field Crops, 4th ed., 1995, pages 132-155) ("Poehlman"), and in view of Cheng *et al.* (*Plant Physiol.* 115: 971-980, 1997) ("Cheng"). In particular, it is asserted that Bowen teaches a method of producing transgenic cereal plants from a single explant by culturing the explant in a multiple bud inducing media and introducing exogenous DNA into the explant, Zhong teaches an explant presenting a plurality of meristems, Poehlman teaches harvesting shoots and transferring them to a culture medium that promotes root development, Weeks teaches exogenous DNA encoding a protein conferring resistance to a selection agent, and Cheng teaches transforming wheat. Thus, the Action finds that it would have been obvious to combine the references to arrive at the invention. Applicants respectfully traverse.

Applicants note that the claimed invention is directed to "culturing…explant in a…multiple bud inducing media suitable for inducing production of a plurality of *buds* from at least one of said meristems" [emphasis added], and "introducing exogenous DNA into more

than one of said plurality of *buds*" (claim 1(b-c) [emphasis added]). Although the Action asserts at page 4 that Bowen teaches the elements of claim 1, parts b-c, Applicants respectfully submit that Bowen does not teach or suggest all of these claimed features. In particular, the Action at page 4 relies on Bowen, column 2, lines 24-51 including lines 45-46, in asserting that Bowen teaches introducing exogenous DNA into a plurality of **meristems** (emphasis added). Likewise, FIG. 2 of Bowen describes meristem transformation followed by later steps that may include shoot multiplication. However, present claim 1(c) does not recite introducing DNA into **meristems**. Instead, present claim 1 (c) relates to introducing DNA into a plurality of **buds**. Applicants note that meristems and buds are not equivalent structures. For instance, buds are differentiated, while meristems are not. Thus, the transformation targets here (*i.e.* cells to be transformed) of Bowen are not the same as the transformation targets of the present application. As distinct from the aspects of Bowen cited here, the present application teaches that meristems may be cultured to induce multiple buds, which may then be transformed by exogenous DNA.

Further, regarding claims 11 and 15, the Action at page 5 relies on col. 19, lines 41-46, of Bowen to assert that Bowen teaches introducing exogenous DNA. However, this relied-upon portion and lines above and below this relied-upon portion at col. 19, lines 19-55 assert as follows:

> (B) Staging and Selection of Responsive Explants
>
> The size of the whole excised ear and the developmental stage of the meristems were found to be reliable indicators of proper timing of harvest…The upper limit for selection of responsive targets was determined by *meristem stage*; developmental plasticity decreased dramatically once the glumes began to be obvious and approached the sides of the *meristematic dome*.
>
> (C) Initial Culture Medium
>
> Various media have been used, and inbreds respond differently to these variations. *A preferred medium used in the initial stage of floral meristem culture*

(used for various genotypes) consisted of Murashige and Skoog salts, MS vitamins, 0.1 mg/l 2,4-D, 0.5 gm/l6-BAP, l-proline at 12.2 μM, 8% sucrose, and silver nitrate at 30 mg/l. A preferred gelling agent is GELRITE (product of Merck and Co, Inc./Kelco division, Rahway, N.J.) at 3.5 g/l.

(D) Bombardment

Immature ear explants were bombarded using 650 psi rupture disks and a stainless steel screen (100 um mesh size) suspended approximately 0.5 to 1.0 cm above the tissue. DNA precipitation and other bombardment parameters were as described in Example 1.

(E) Subsection, Subculture and Selection

Maintenance of rapid growth and *survival of individual meristems was achieved by subsecting the ears four to six days after isolation, into pieces with four to eight meristems each*. These pieces were cultured onto shoot multiplication medium, which has the same basal composition as the initial culture medium (above) but with 1 mg/l BAP and 3% sucrose. Meristem tissue was subcultured repeatedly, at two week intervals on the shoot multiplication medium.

See col. 19, lines 19-55 of the specification. [emphasis added]

Accordingly, the explants used in Bowen are meristems, which are still earlier stage (meristem stage) precursor tissues and have not yet differentiated into later stage bud tissues. That is, DNA is bombarded into the cells of these earlier stage precursor tissues (*i.e.*, meristems), not into buds. In contrast, the claimed invention is directed to introducing DNA into buds that are no longer meristems, having already differentiated from their respective meristems.

Furthermore, since the Bowen reference relates only to meristems as discussed above, the media used therein is a *meristem culture media* suitable for producing meristem explants so as to introduce an exogenous DNA into the cultured meristems. In contrast, the claimed invention is directed to "a *bud inducing media* suitable for inducing production of a plurality of buds from at least one of said meristems." [emphasis added].

Regarding claim 13, the Action at page 5 again relies on Bowen, column 2, lines 24-51, in asserting obviousness. Applicants note that claim 13 is an independent claim, and no other

reference is being cited in the rejection of claim 13. Thus the rejection might be more properly framed as an anticipation rejection. At any rate, Applicants respectfully traverse the rejection as follows:

Applicants note that the Action at page 6, explicitly concedes that Bowen does not teach use of wheat mesocotyl explants (regarding claim 8), or wheat mesocotyl explants presenting a plurality of meristems (presumably regarding claim 13). Claim 13 however recites that wheat mesocotyl explants are to be utilized. Thus, the rejection at least of claim 13 in view of Bowen is mistaken, in that not all of the limitations of claim 13 are taught by Bowen, and no other references are cited in regard to claim 13. Applicants note also though, that in the rejection of claim 8, Zhong is asserted to teach use of maize mesocotyl explants, and that it is asserted that it would have been obvious to adapt a method of wheat transformation in view of the teachings of Zhong and Bowen, as both maize and wheat are monocotyledonous plants. Thus, in the interest of furthering prosecution, a response to the rejection of claim 13 is also made in view of the Zhong reference, and Applicants further submit that this reasoning is also relevant to the asserted rejection of claims 7 and 8, as well as the rejection of claims 14-16 which depend from claim 13. Further, as noted below, Applicants' comments regarding Zhong are similarly relevant in view of its use in the rejection of claim 1 (as teaching step (a)).

A careful reading of Zhong, as cited at page 483, 2nd column, 2nd paragraph, indicates that Zhong is not describing use of a **mesocotyl explant** (*e.g.* claim 8) or wherein the meristems contain a "**scutellar node**" (*e.g.* claim 7). Instead, Zhong is describing identification and excision of a **shoot apex** of a recently germinated seed, in part by using the presence of the mesocotyl as a positional marker. For instance, at p. 383 of *Plant Physiology*, 3rd ed., by Salisbury and Ross, which was provided as an appendix to the previously filed

Appeal brief, Figure 19-7 shows morphological characteristics of a week old maize seedling, comparable to the tissues being described in Zhong. The mesocotyl is described in Salisbury & Ross as being the internode formed above the seed storage tissues (including scutellum). Here the monocot shoot apex is described as being at the node where adventitious roots originate, while the mesocotyl extends between the scutellum (cotyledon) and that node. However, Zhong teaches excision of "(s)ections about 5 mm long of seedlings containing a shoot tip, three to five leaf primordia, and a portion of young leaf and stem immediately below the leaf primordia" (Zhong, last paragraph on p. 483). Zhong does not describe excising the node, or any adventitious roots, let alone any mesocotyl tissue that would be found below the node and adventitious roots. Nor does Zhong contemplate use of the scutellar node which would be "below" the mesocotyl as it is shown in Figure. 19-7. That is, Zhong is apparently only describing excision of a shoot apical meristem, *i.e.* a primary meristem, and is not contemplating use of other meristems such as an axillary meristem associated with the scutellar node. Again, Zhong only mentions mesocotyl to identify the positioning of the tissues taken as an explant, not to teach excision of a mesocotyl explant as presently defined.

However, the present application, for instance at paragraph [0003] bridging pages 1-2, and paragraph [0029] at page 5, explicitly states that the mesocotyl explant being contemplated comprises an apical meristem and axillary meristems, including the scutellar node meristem. Further, "(w)ithin the method of this invention, primary and axillary meristems are induced to generate multiple secondary buds…" (paragraph [0003]). Importantly, an aspect of the present invention relates to using an explant that comprises multiple meristems, both primary and axillary, which may subsequently be cultured. Zhong teaches use of the shoot tip, presumably including a primary (apical) meristem. However, Zhong clearly teaches that other portions of

the germinating seed, including the mesocotyl with its axillary meristems, are to be discarded. Since use of mesocotyl tissue or scutellar node tissue as presently claimed is not equivalent to use of only a shoot tip or shoot apical meristem as described by Zhong, the defect of Bowen relating to the present claim limitations that recite use of mesocotyl tissue or scutellar node tissue has not been cured. Indeed, Zhong **teaches away** from use of mesocotyl tissue, or scutellar node tissue that may contain axillary meristems. Thus, the rejection of at least claims 7, 8, and 13-16 is not supported by Zhong in conjunction with Bowen, let alone by Bowen alone, and withdrawal of these rejections is respectfully requested.

The Action also cited Zhong with regard to claim 1 (step (a)), asserting at page 6 that "Zhong teach claim 1, step (a) providing an explant presenting a plurality of meristems." Applicants respectfully traverse.

Applicants respectfully submit that the explant as described by Zhong in the section bridging pages 483-484 does not inherently comprise multiple meristems. Rather, if multiple meristems are eventually utilized, it is only after subsequent "shoot multiplication" culture of that initial explant tissue. The explant as initially obtained according to Zhong is described in the Action at page 7, lines 2-3, as "a portion of young leaf and stem immediately below the leaf primordial (sic)..." This is apparently coleoptile tissue as shown in Figure 19-7 of Salisbury & Ross, discussed above. Zhong does not describe excision of node tissue, which might be expected to contain axillary meristem(s). Supporting this, Zhong repeatedly describes their explant as "shoot tips" (*e.g.* page 484, 1st paragraph), and a skilled artisan would not understand that a shoot tip explant inherently comprises a node. Thus, Applicants respectfully submit that the Action's use of Zhong to cure Bowen's defect relating to claim 1, step (a), (as well as claim 13) is mistaken, and if anything, Zhong **teaches away** from the presently claimed

methods. As no other references, *e.g.* Weeks, Poehlman, and Cheng, are cited that cure the defects of Bowen relating to claim 1, step (a), and/or claim 13, withdrawal of the rejection of claims 1-16 is respectfully requested.

Additionally, the claimed invention yielded surprising, unexpected, and unpredictable results in light of the cited references or the art in general. Specifically, the claimed method yields production of **multiple** secondary buds. *See* Specification, paragraph [0052]. These secondary buds may then be used as a target for transformation, thus increasing the number of target cells per explant, allowing for an increased output of transformed material without significantly increasing the amount of labor or plant tissue input. These results are significant in that they represent a potential increase of orders of magnitude in the number of transformed wheat plants obtained, on a per explant basis. In contrast, the Bowen reference asserts that its method produced GUS frequency of 3%-34%, and further asserts that only 2 transgenic plants out of 48 bombarded embryos survived. *See* col. 20, Table 4 and col. 21, lines 42-47 of Bowen. In contrast to Bowen, Table 5 of the Specification shows that the present method can result in 49-93 buds (and hence potentially transformed plants) *per explant*, while Table 6 shows that even with cv. Autry, the least efficient cultivar in Table 6, more than 60% of explants could produce more than 20-80 buds per primary meristem in an explant, again demonstrating a significant improvement in the ability to efficiently regenerate wheat plants following transformation procedures. Table 8 shows that 20-48 elongated shoots could be produced, again *per explant*. Tables 11 and 13 likewise demonstrate that as high as 34.7% of explants could produce stably transformed glyphosate tolerant plants, again at least an order of magnitude improvement over the wheat transformation described in the art. Thus, the instant invention surprisingly yielded a substantial and unexpected increase in the efficiency of the

transformation process. As such, removal of the rejection of claims 1-16 is respectfully requested.

2. Rejection Over Fry *et al.* in view of Eudes *et al.*

The Action rejects claims 1-16 under 35 U.S.C. § 103(a) as being obvious over Fry *et al.* (U.S. Patent 5,631,152) ("Fry") in view of Eudes *et al.* (U.S. Patent 6,995,016) ("Eudes"). Specifically, the Action asserts that Fry teaches "a method for producing transgenic wheat comprising culturing explants, introducing exogenous DNA *via* bombardment, transferring cells from a first media to a second media to induce elongation of buds into shoots, harvesting and transferring shoots to a culture medium that promotes root development, and culturing the transferred shoots to produce plants." The Action further asserts that Eudes teaches a multiple shoot induction media containing cytokinin. Thus, the Action finds that it would have been obvious to combine the references to arrive at the invention. Applicants respectfully traverse.

Claim 1 of the instant invention recites that the explant is to be cultured "in a first *multiple* bud inducing media suitable for inducing production of a plurality of buds from at least one of said meristems" [emphasis added]. Fry does not teach or suggest culturing an explant on a multiple bud inducing media. Rather, Fry refers to a tissue culture method wherein "the regenerable plant tissue is placed in a medium capable of producing shoots from the regenerable tissue" (col. 4, lines 35-39), and then transferred to a second medium capable of producing roots from said shoots (col. 5, lines 22-23). The regeneration of a plurality of buds from *multiple explants*, as referred to in Fry, is not the equivalent of regeneration of a plurality of buds from a single explant. As noted in the specification, "the primary meristems give rise to multiple secondary buds, in some cases upwards of one hundred secondary buds per primary meristem" (paragraph [0051]). This "allows for an increased output without

significantly increasing the amount of labor or plant tissue input" (see paragraph [0051]). There is no teaching in Fry of a method involving placing the explant on a media suitable for regeneration of multiple buds from a single primary meristem. Therefore, Fry does not teach all elements of the claimed invention.

Eudes does not cure the defects in the Fry reference. Eudes is apparently added in view of its discussions relating to uses of hormones in plant cell culture. However, Eudes relates only to culturing *immature scutella* cells from embryos or callus through direct or indirect embryogenesis (Eudes, col. 7, lines 11-14, although a callus stage may also be utilized, but still requiring embryogenesis; *see also* Eudes col. 8, lines 34-49). At page 12, the Action describes Eudes do not teach away from the presently claimed subject matter because Eudes *et al* teach (use of) a medium that contains both a cytokinin and an auxin (*e.g.* Eudes, column 5, lines 12-14). However, Applicants note that the media being discussed there by Eudes is a *callus induction* medium, indicative of an embryogenic approach for (eventual) creation of transformed plants. That is, undifferentiated callus is grown, which is later cultured so that embryos arise (embryogenesis), which then give rise to differentiated plant organs such as buds and shoots,

As discussed extensively in prior prosecution, embryogenesis (even if later followed by organogenesis) represents an approach for wheat culture and transformation that is distinct from the bud-forming (*i.e.* initially organogenic*)* approach described in the present application. Thus, Eudes is **teaching away** from the present method regardless of the ingredients of any culture medium being described therein. Additionally, a skilled worker would have **no expectation of success** in utilizing these teachings of Eudes, which relate to an embryogenic

culture approach, when attempting to grow multiple transgenic wheat plants by utilizing the presently claimed inherently organogenic approach, as also discussed below.

Applicants further note that Eudes mentions an organogenic approach in the Background of Invention section (Eudes, column 3, line 23 and following), however wherein organogenesis is explicitly described as comprising "...the development of...(o)nly one meristem..." (Eudes, col. 3, lines 29-31). Eudes also describes an organogenic approach in their Detailed Description (*e.g.* col. 15, line 59 and following). However, this approach requires prior embryogenesis. Further, Eudes does not suggest wheat in this context. Thus, Eudes apparently does not recognize that multiple meristems or additional buds may be formed in the presence of a cytokinin, in wheat. Eudes is therefore not properly applied.

Additionally, Eudes asserts that embryogenesis is preferred over organogenesis. (*e.g.* Eudes, col. 3, lines 48-62). This clearly **teaches away** from the present invention, which instead relates to manipulation of explants comprising meristems without the presumed disadvantages of an embryogenic approach which were known to include, for instance, genotype dependence, somaclonal variation, poor regeneration, and/or reduced fertility. Specification, *e.g.* paragraph [0028]. If anything, a skilled worker would conclude, after reading Eudes, that the presently claimed approach would **not** be expected to yield efficient methods for producing transgenic wheat plants, *i.e.* there would be **no reasonable expectation of success** in combining the teachings of Fry and Eudes in pursuing a non-embryogenic approach for obtaining transgenic wheat plants.

As explained above, the claimed method yields production of multiple secondary buds (*see* Specification, paragraph [0052]). These secondary buds may then be used as a target for transformation, thus increasing the number of target cells per explant, allowing for an increased

output of transformed material without significantly increasing the amount of labor or plant tissue input. These results are significant in that they represent a potential increase of orders of magnitude in the number of transformed wheat plants obtained, on a per explant basis. In contrast, the cited Fry reference states, at Table 2, that use of the "rapid" technique resulted in a transformation frequency of bout 1.3%. That is, of 2675 embryos subjected to transformation, 36 glyphosate tolerant plants were recovered. In contrast to Fry *et al.*,, as discussed above, Table 5 of the Specification shows that the present method can result in 49-93 buds (and hence potentially transformed plants) *per explant,* while Table 6 shows that even with cv. Autry, the least efficient cultivar in Table 6, more than 60% of explants could produce more than 20-80 buds per primary meristem in an explant, again demonstrating a significant improvement in the ability to efficiently regenerate wheat plants following transformation procedures. As discussed above, Table 8 shows that 20-48 elongated shoots could be produced, again *per explant* and Tables 11 and 13 likewise demonstrate that up to 10-30% of explants could produce stably transformed glyphosate tolerant plants, again at least an order of magnitude improvement over the wheat transformation described in the art. The present method thus allows a substantial and unexpected increase in the efficiency of the transformation process.

At page 13, the Action cites MPEP 2144.09 in rejecting the Applicants' assertion of unexpected results. Applicants respectfully traverse, and submit that this citation is inapposite. In particular, MPEP 2144.09 relates specifically to chemical compounds (*i.e.* compositions). However, no compositions are being claimed here, only methods. Even if some bud inducing medium were considered structurally similar to one found in the prior art, **no bud inducing medium (composition) is being claimed here** in spite of the assertion in the Action at page

13. Rather, methods of culturing wheat tissues, to produce transgenic plants, are being claimed. Withdrawal of the rejection is thus respectfully requested.

At page 14, the Action asserts that one of ordinary skill in the art would recognize cytokinins would be useful because Eudes teach that cytokinins are useful in cell division. Applicants traverse, and note, again, that this is only a most general teaching, and Eudes is discussing an embryogenic approach for creating cultured wheat plants. Since organogenesis and embryogenesis are developmentally distinct, a skilled artisan **would not apply, with any expectation of success**, conditions for embryogenic culture while trying to achieve organogenesis.

At page 15, the Action also asserts that one of ordinary skill in the art would understand that mesocotyl tissue can be used in the methods taught by Fry *et al.* and Eudes *et al.* Applicants respectfully traverse. Although Fry is asserted as teaching that any regenerable plant tissue can be used, mesocotyl tissue is not discussed by Fry. The cited teaching of Fry is only of the most speculative, cursory and general sort, and would **not give a skilled artisan any expectation of success**, among numerous experimental variables relating to potentially regenerable plant tissues that might be utilized. The Action thus displays hindsight reasoning in making this argument. Furthermore, the enhanced efficiency of transformation (*e.g.* number of transformed plants) demonstrates **unexpected results** when the presently claimed method, utilizing for instance, mesocotyl tissue, is followed. In view of the foregoing, withdrawal of the obviousness rejection is respectfully requested.

C. Rejection for double patenting

The Action next rejects claim 1 on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1 and 7 of U.S. Patent No. 5,631,152 (Fry *et al.*). As discussed above, the current application is not made obvious by Fry *et al.* There is no teaching in Fry of a method involving placing the explant on a media suitable for regeneration of multiple buds from a single primary meristem. As noted above, the regeneration of multiple plants from multiple explants, as referred to in Fry, is not the equivalent of regeneration of multiple buds from a single explant. Additionally, Fry does not teach or suggest the surprising and unexpected results obtained with the methods of the instant invention, namely obtaining multiple transgenic plants from a single explant by virtue of a multiple bud-inducing media. As Fry does not teach or suggest a medium for inducing the production of a plurality of buds from a single explant, the reference does not create a *prima facie* case of obviousness and cannot be the basis for an obviousness-type double patenting rejection. In light of the foregoing, Applicants respectfully request withdrawal of the rejection.

D. Conclusion

In view of the above, it is submitted that all of the rejections to the claims have been overcome, and the case is in condition for allowance.

The Examiner is invited to contact the undersigned at (214) 259-0931 with any questions, comments, or suggestions relating to the references patent application.

Respectfully submitted,

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